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# Isolation and identification of symbiotic strains in *Paris fargesii Franch* that promote disease resistance

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*Pairs polyphylla* var. *yunnanensis* is a perennial herb, and its rhizomes are used as medicine to invigorate blood circulation, dissipate blood stasis, relieve swelling and pain, clear heat and detoxify the body. However, due to its slow growth, most of the saponin content is low, while the content of saponins is higher in *Paris fargesii Franch* of the same genus. The isolation and screening of strains from *P. fargesii Franch* that promote the accumulation of saponin content in *P. polyphylla* var. *yunnanensis* is important to improve the growth rate of artificially cultivated *P. polyphylla* var. *yunnanensis* and increase its medicinal activity. Plant symbiotic strains are biologically active microorganisms that can promote the growth and development of host plants through direct probiotic effects and indirect disease resistance mechanisms. Here, we isolated four strains of symbiotes, including two bacteria, *Bacillus cereus* (LgD2) and *Bacillus thuringiensis* (LgD10), and two fungi, *Aspergillus tamari* (TPD11) and *Fusarium oxysporum* (TPB). All of them promoted the biomass, chlorophyll content and photosynthetic efficiency of *P. polyphylla* var. *yunnanensis* seedlings to different degrees and effectively stimulated the accumulation of saponin content in *P. polyphylla* var. *yunnanensis* seedlings to improve the expression of saponin synthesis pathway genes. The symbiotes also promoted the enzymatic activities of the enzymes superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine aminolase (PAL), improved the physiological indicators of tobacco plants (plant height, crown width, leaf length and leaf width), reduced the incidence of tobacco black shank, and improved the growth and resistance-related genes of tobacco. In conclusion, the four symbiotic strains TPD11, TPB, LgD10 and LgD2 play an important role in regulating the growth, promoting saponin accumulation, and disease resistance of *P. polyphylla* var. *yunnanensis*.

## KEYWORDS

*Paris fargesii Franch*, tobacco, steroid saponin, symbiotic strains, tobacco black shank

## 1 Introduction

*Pairs polyphylla* var. *yunnanensis* is a perennial herb of the genus *Paris* in the family Trillaceae and is used as a medicine with rhizomes to activate blood circulation and disperse blood stasis, reduce swelling and pain, cool the liver and calm the panic, clear heat and detoxify the body. *P. polyphylla* var. *yunnanensis* has anticancer and antibacterial effects (Li et al., 2014; Wen et al., 2015) and is currently the main ingredient of some important Chinese patent medicines, such as Yunnan Baiyao, Gonghening capsules, and Sichuan Baiyao (Wu et al., 2004). Steroidal saponins are the main active medicinal ingredients of bryophyllum, including polyphyllin I, polyphyllin II, polyphyllin VI, polyphyllin VII, polyphyllin D, and polyphyllin H (Zhao et al., 2009; Zhao et al., 2017). Successive generations of the Chinese Pharmacopoeia have included *P. polyphylla* var. *yunnanensis* as a traditional Chinese medicine. With the deep development of the medicinal value of *P. polyphylla* var. *yunnanensis*, market demand is rising year by year. However, due to years of slow growth and overharvesting, the regeneration of wild resources of *P. polyphylla* var. *yunnanensis* has been seriously damaged, and the current cultivation of *P. polyphylla* var. *yunnanensis*, because its long growth cycle (8-10 years) is unsatisfactory, and its saponin content is low, is often unable to meet the needs of pharmaceutical companies. However, *Paris fargesii* Franch is a sibling species of *P. polyphylla* var. *yunnanensis* and has a high saponin content (Wen et al., 2019). Therefore, it is important to isolate and identify symbiotic strains from *P. fargesii* Franch and screen them for the growth and accumulation of saponins to improve the growth rate of *P. fargesii* Franch. It is important to improve the growth rate and increase the accumulation of medicinal active ingredients in artificial cultivation.

Symbiotic strains are a group of microorganisms that can achieve a symbiotic relationship with plants during plant growth and in the environment, mainly including endophytes and rhizospheres. Symbiotic strains can promote plant growth through direct and indirect mechanisms (Glick, 1995): direct mechanisms are manifested in the ability of the strain to promote nutrient uptake by the host plant through its own nitrogen fixation (Pérez-Montañó et al., 2014), phosphate solubilization (Alori et al., 2017), potassium solubilization (Zhang et al., 2020) and other probiotic characteristics, providing vitamins needed for plant growth, and through the

**Abbreviations:** PPO, Polyphenol oxidase; PAL, Phenylalanine aminolase; SOD, Superoxide dismutase; POD, Peroxidase; PDA, Potato Dextrose Agar (Medium); LB, Luria-Bertani (Medium); Pn, Net photosynthetic rate; Gs, Stomatal conductance; Ci, Intercellular CO<sub>2</sub> concentration; Tr, Transpiration rate; HPLC, High-performance liquid chromatography; HMGS, Hydroxymethylglutaryl coenzyme A synthase; GPPS, Geranyl diphosphate synthase; FPPS, Farnesyl pyrophosphate synthase; SS, Squalene synthase; SE, Squalene epoxidase; CAS, Cycloartenol synthase; IAA, Indole acetic acid.

production of growth hormone-like phytohormones (Ahmed and Hasnain, 2010; Wagi and Ahmed, 2019) similar to the phytohormones of the host plant. A symbiotic strain also promotes the accumulation of active substances in the host plant by producing some physiologically active metabolites that are the same as or similar to the metabolites of the host plant, such as paclitaxel (Stierle et al., 1993), podophyllotoxin (Eyberger et al., 2006), camptothecin (Kusari et al., 2009), huperzine (Li et al., 2007) and quercetin (Min et al., 2010); the indirect mechanism is manifested mainly in the ability of symbiotic strains to directly inhibit the growth of pathogenic bacteria (Zhang et al., 2017) and to enhance host plant biocontrol enzymes such as polyphenol oxidase (PPO), phenylalanine aminolase (PAL), superoxide dismutase (SOD) and peroxidase (POD) active enzymes (Sang et al., 2019) and the expression of related resistance genes (Sherameti et al., 2005), thus indirectly promoting plant growth.

Tobacco black shank is a serious tobacco disease caused by the soil-borne fungus *Phytophthora nicotianae*. The severity of tobacco black shank disease is increasing every year, causing significant economic losses. Chemical fungicides such as methomyl, carbendazim and daidzein are commonly used in the field for disease control, relying on chemicals and leaving fungicide residues causing environmental pollution. Biological control provides a safe alternative to control tobacco black shank disease (Edlayne et al., 2009; Ji et al., 2014; Guo et al., 2020; Ding et al., 2021). Currently, some plant symbiotic strains, such as *Bacillus* spp., *Trichoderma* spp. and *Pseudomonas* spp., have been demonstrated to be useful as biocontrol agents for the suppression of tobacco black shank disease (Cui et al., 2014; Ma et al., 2018; Guo et al., 2020). Therefore, the aim of this study was to isolate and identify symbiotic strains of *P. fargesii* Franch, to evaluate their growth-promoting properties and pathogenic antagonistic activity, and to identify strains that could promote the growth and saponin content accumulation of *P. fargesii* Franch and antagonize the tobacco black shank disease.

## 2 Materials and methods

### 2.1 Plant materials

Four-year-old *P. fargesii* Franch and two-year-old *P. polyphylla* var. *yunnanensis* seedlings were purchased from Wuding County, Yunnan Province, China, and samples attached to the roots were collected from the top five centimeters of soil around healthy *P. fargesii* Franch plants. The collected soil samples (20) were sealed in sterile polyethylene bags and transferred to ice boxes for transport. Plant and soil samples were preserved at the Yunnan Agricultural University Laboratory, with follow-up experiments. For endophyte isolation, healthy *P. fargesii* Franch rhizomes (20) collected from the samples described

above were packed in polyethylene bags, labeled and stored in the laboratory in a 4°C refrigerator and processed within 48 h of collection. The seeds of the disease-susceptible variety of tobacco Hongda were purchased from the Yunnan Provincial Tobacco Monopoly Bureau, and the tobacco seeds were grown in seedling trays until the seedling stage and then transplanted to the field for the trial. The experimental site was in the greenhouse of Yunnan Agricultural University.

## 2.2 Isolation of endophytic and interrhizosphere bacteria

### 2.2.1 Isolation of endophytic bacteria

The harvested healthy *P. fargesii* Franch was rinsed with running tap water, selected healthy tissues were cut into small sections, and the surface water was blotted with filter paper. The samples were soaked in 75% ethanol for 1 min, rinsed in sterile water 3 times, soaked in effective chlorine 5% sodium hypochlorite solution for 3 min, and finally washed in sterile water 3-4 times until the disinfection solution was washed off thoroughly. The different tissues of Rhizoma Paridis were ground in a sterilized mortar, mixed with sterile water and transferred to an Eppendorf (EP) tube containing 5 ml of Luria-Bertani (LB) liquid medium; the shaker was set at 37°C and 180 rpm and shaken for 2-3 h. The suspension was then diluted to three different concentration gradients of  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ; 100  $\mu$ l of the above suspension was applied to LB plate medium, and 100  $\mu$ l of sterile water from the last wash was used as a control. Three replicates of each gradient were set up and incubated in an inverted incubator at 37°C for 24-48 h. When bacterial colonies grew, single colonies with different characteristics of morphology and color were selected, scraped onto LB solid culture plates with an inoculation loop for delineation, and numbered. After several rounds of delineation and purification, the isolated bacterial strains were stored at -80°C at a ratio of 1:1 bacterial solution and 50% sterilized glycerol.

### 2.2.2 Isolation of endophytic fungi

The freshly isolated rhizomes of *P. fargesii* Franch were rinsed with tap water. The samples were rinsed with 70% ethanol for 1 min, followed by 5 repeated rinses with sterile water and then disinfected with 5% NaClO for 5 min, followed by 5 repeated rinses with sterile water and blotting with sterilized filter paper to remove excess water. Finally, the rootstock was cut into small pieces with a sterilized blade. Potato dextrose agar (PDA) medium (with streptomycin) was prepared, and small segments of the roots were placed in the medium with 4 pieces of tissue in each Petri dish and incubated at a constant temperature of 30°C. Then, we inoculated 100  $\mu$ l from the last wash of sterile water onto PDA plates for surface sterilization examination of the plant tissue samples. Promptly after 5-10 d of incubation, we used the tip mycelium picking method to pick morphologically

different mycelia or colonies to transfer to new plates. After 5-10 d of incubation, we picked mycelia or colonies with different morphologies, transferred them to fresh PDA medium for purification, and then transferred them to agar slants for storage.

### 2.2.3 Isolation of interroot bacteria

The collected soil samples were sieved to remove the large hard particles and nonsoil materials. The soil suspension was made as follows: 10 g of soil was weighed and placed in a culture bottle with 90 ml sterile water, and the bottle was sealed with film. The bottle was placed in a shaker at 37°C and 180 rpm for 2 h to make a soil suspension. The bottle was removed and left for 5 min to precipitate the large particles, and 2 ml of soil suspension was pipetted into a 2.5-ml sterilized centrifuge tube with a 1000-ml pipette. After a short centrifugation, 1 ml of supernatant was taken and added to a test tube with 9 ml of sterile water, shaken back and forth 5 times, mixed well, marked as  $10^{-2}$ , and diluted into  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  gradient dilutions. For the coating plate method culture, we used a 100- $\mu$ l pipette, aspirated 80  $\mu$ l dilution from the 4 dilution tubes, added it to LB medium, used an applicator to coat the dilution evenly on LB medium, sealed the tube, and placed it in a 30°C incubator inverted culture for 1-2 d. We repeated each gradient three times, set a blank control at the same time; waited for the bacterial colonies to grow out, selected the different morphology and color different characteristics of the single colonies, scraped the single colonies onto LB solid culture plates with an inoculation ring for scribing and numbering, respectively; after purification of the bacterium, it was placed in shaking culture with 1.5 ml of liquid LB into bacterial solution. We aspirated 1 ml of bacterial solution, 50% sterilized glycerol was added a ratio of 1:1, and the sample was stored at -80°C.

### 2.2.4 Isolation of interroot fungi

One gram of the raw interroot soil material was weighed for the experiment, and 90 ml of sterile water was added to a 100-ml conical flask. We prepared PDA medium (plus penicillin) in 10 Petri dishes and took 100  $\mu$ l of bacterial suspension coated on PDA plate medium, set 5 gradients, each gradient with 2 replicates, placed the samples in a 25°C incubator for incubation for 24-48 h. We observed different colors and shapes to screen the colonies, distinguished different colors and sizes of the single colonies, separated and grew them until there was only one kind of bacteria in the medium after purification.

## 2.3 Amplification and sequencing of bacterial 16S rRNA and fungal internal transcribed spacer

The bacterial 16S rRNA gene and fungal ITS rDNA were amplified and sequenced for molecular identification. Endophyte genomic DNA was extracted using the

cetyltrimethylammonium bromide (CTAB) method (Kosecka-Strojek et al., 2019). The primer sequences used for PCR identification were 27F (5'-GAGTTTGA TCACTGGCTCAG-3'), 1492R (5'-TACGGCTACCTTGTTACGA-3') ITS1 (5'-TCCGTGGACTGACTCGG-3') and ITS4 (5'-TCCTCGG-CTTA TGA TGC-3') (Hassan, 2017). The polymerase chain reaction (PCR) system contained 22.5  $\mu$ l of Gold MIX, 1  $\mu$ l of each primer, and 0.5  $\mu$ l of genomic template. The PCR amplification system was as follows: 98°C predenaturation for 2 min, 98°C denaturation for 10 s, 55°C annealing for 30 s, 72°C extension for 10 s for 30 cycles followed by 5 min extension at 72°C. After the reaction, the products were loaded on a 1% agarose gel and electrophoresed at 150 V for approximately 20 min. The amplification effect was detected by electrophoresis, and the quality was assessed according to the test results and sent to Kunming Shuokeng Biotechnology Co. The sequencing results were compared with basic local alignment search tool (BLAST) in the National Center for Biotechnology Information (NCBI) database, and homologous sequences were searched to find the strains with the highest similarity and determine their biological classification status.

## 2.4 Growth-promoting biological activity of the strains

The nitrogen fixation capacity of the symbiotic strains was tested on a modified low nitrogen medium (Ogura-Tsujita and Okubo, 2006), the potassium solubilization capacity of the symbiotic strains was tested by the method of Zheng et al. (Li et al., 2019), the phosphorus solubilization capacity of the symbiotic strains was tested by the method of Jasim et al. (2013), and the indole acetic acid (IAA) production capacity was determined by the Salkowski color development method, which was developed at room temperature in the dark for 20 min. The appearance of a pink color was positive, indicating the presence of IAA production, and the darker the color, the stronger the ability to produce IAA (Chen et al., 2019). The absorbance of the mixture was calibrated with a spectrophotometer at 530 nm using the same solvent calibration (control), and the concentration of IAA production by the strain was determined using the calculated standard curve  $y=40.063x-1.9228$  ( $R^2 = 0.9469$ ).

All the isolated symbiotic strains were antagonistic with *Phytophthora nicotianae* to screen for antagonistic strains of the symbiotic strains. The pathogen was inoculated in the middle of a Petri dish containing PDA medium, and the isolated and purified symbiotic strains placed on the side of the pathogen were recorded as the treatment group. A control of *Phytophthora nicotianae* alone was set up and incubated in an incubator at 28°C for more than 7 d. Each treatment was repeated three times. After stand-off culture for more than 14 d, the growth of the pathogenic fungus was observed, and

pathogenic fungi covering the strain growth were not inhibited. The radius of the control pathogenic fungus with inhibitory effects and treatment group pathogenic fungus size were measured, and the inhibition rate was calculated according to the size of the inhibition rate to determine the antagonistic ability of the fungus. Inhibition rate (100%) = (control colony radius - treatment colony radius)/control colony radius x 100%.

## 2.5 Promotional effect of symbiotic strains on the seedlings of *P. polyphylla* var. *yunnanensis*

### 2.5.1 Inoculation of *P. polyphylla* var. *yunnanensis* seedlings with symbiotic strains

Preparation of symbiotic strain suspension: On an ultraclean bench, we aspirated 1 ml of purified symbiotic strains into a triangular flask containing 500 ml of autoclaved LB liquid medium, sealed the flask, incubated it at 200 rpm at 37°C until the OD600 was 0.5-0.6, centrifuged it at 5000 rpm for 5 min in a 500 ml sterile centrifuge tube, and resuspended the bacteria in 500 ml of sterile water. Preparation of the spore suspension of symbiotic fungi for watering: Five hundred milliliters of PDB liquid medium was poured into a culture bottle sterilized by autoclaving at 121°C in an aseptic environment. Then, fresh mycelium was picked, placed in a culture bottle and sealed. After 200 rpm·min<sup>-1</sup> and 30°C shock incubation for 6 d, the effective live count of the liquid was determined to reach 1×10<sup>8</sup> CFU/ml, and then it was used for watering. In September 2020, two-year-old seedlings of *P. polyphylla* var. *yunnanensis* were transplanted, and five months after transplanting, four strains were used to irrigate the *P. polyphylla* var. *yunnanensis* in the form of a root suspension, and sterile water was used as the control, with a total of five treatments. Each treatment was planted in six pots, and each pot was planted with 10 two-year-old seedlings of *P. polyphylla* var. *yunnanensis*, and each pot was irrigated with 50 ml of bacterial suspension. After a total of 60 d of incubation, the rhizomes were removed from the roots, washed with water, and blotted with wiping paper. The following data were statistically analyzed.

### 2.5.2 Analysis of agronomic traits and chlorophyll and photosynthesis parameters

The natural plant height, root length, leaf length and leaf width of the seedlings were measured with a straight edge (accuracy 0.1 cm); the fresh weight root weight and aboveground fresh weight of the seedlings were weighed accurately with an electronic balance (accuracy 0.001 g). Fresh leaves were taken, the midvein was removed, and 0.1 g of sample was weighed and placed in a mortar. We added 1 ml of distilled water, a small amount (10 mg) of reagent one, ground it thoroughly under low light conditions, and transferred it into

a 10-ml test tube wrapped with aluminum foil. We rinsed the mortar with the extraction solution, transferred all the rinsing solution into a 10-ml test tube, adjusted the volume to 10 ml with the extraction solution, and placed it under dark conditions for 3 h. When we observed that the tissue residue at the bottom was close to white, we considered the extraction complete. The absorbance values at 663 nm and 645 nm were measured by taking 1 ml of the upper layer of the extract in a glass cuvette and recorded as  $A_{663}$  and  $A_{645}$ , respectively. The gas exchange parameters of the functional leaves of the heavy plant were measured using a Li-6400 XT photosynthesis-fluorescence recorder (Li-Cor, Lincoln, NE, USA) with a red and blue light source leaf chamber, setting a light quantum flux density of 300  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ , a temperature of 25°C, and a  $\text{CO}_2$  concentration of 400  $\mu\text{mol}/\text{mol}$ . The measurement time was chosen from 9:00 to 11:00 in clear weather, and seven representative *Dianthus* seedlings were selected for measurement in each treatment, with one leaf per plant randomly selected. The four indices of the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular  $\text{CO}_2$  concentration (Ci), and transpiration rate (Tr) were measured.

### 2.5.3 Saponin content determination

The treated rhizomes were collected, washed, dried at 45°C to a constant weight, and crushed. We weighed 0.5 g of the crushed sample (sieve No. 3, 50 mesh) into a 50 ml volumetric flask, added methanol and fixed the volume to 20 ml, weighed the sample, soaked it for 30 min, extracted it with ultrasound for 30 min, cooled it at room temperature, made up the lost weight with methanol, shook it well, and filtered the extract with a 0.45- $\mu\text{m}$  microporous membrane. A total of 700  $\mu\text{l}$  was placed into a brown injection vial, and the sample was injected into the machine. The content of steroidal saponins in the rhizomes of the seedlings was determined by high-performance liquid chromatography (HPLC) on an Agilent (Santa Clara, CA, USA) 4.6 $\times$ 250 mm column with the following chromatographic conditions: mobile phase acetonitrile (A): water (B) (gradient elution, 0 $\rightarrow$ 40 min (A: B=30:70 $\rightarrow$ 60:40), 40 $\rightarrow$ 41 min (A:B=60:40 $\rightarrow$ 80:20), 41 $\rightarrow$ 44 min (A:B=80:20 $\rightarrow$ 80:20), (A:B=80:20 $\rightarrow$ 80:20); detection wavelength of 203 nm, column temperature of 30°C, flow rate of 1.0 ml/min, injection volume of 20  $\mu\text{l}$ .

### 2.5.4 Fluorescence quantification of steroid saponin synthesis pathway-related genes

Total RNA was extracted from the rhizomes of the Paris Rhizome using the Polysaccharide Polyphenol Plant Total RNA Kit according to the instructions, and the extracted total RNA of the qualified sample was used for reverse transcription synthesis of first-strand cDNA. cDNA synthesis was performed using Abm's 5 $\times$  All-in-One RT MasterMix kit. The EvaGreen 2X qPCR MasterMix (abm) kit was used to validate the expression levels of seven genes related to the steroid saponin

synthesis pathway: hydroxymethylglutaryl coenzyme A synthase (HMGS), geranyl diphosphate synthase (GPPS), farnesyl pyrophosphate synthase (FPPS), squalene synthase (SS), squalene epoxidase (SE), and cycloartenol synthase (CAS). The primers for the *Actin*, *SE1*, *SE2* gene were designed by the method of Xu Yan et al. (Xu et al., 2017) and the primers for the *FPPS*, *SS*, *CAS*, *GPPS*, *HMGS* gene were designed by the method of Xu Yongyan (Xu, 2017) because the *P. polyphylla* var. *yunnanensis* genes have not been published. All primers were ordered from Tsingke Biotechnology Co., Ltd. (Beijing, China). (Table 1). The PCR conditions were as follows: enzyme activation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and storage at 4°C after 40 cycles. The relative quantification of gene expression was performed using the  $2^{-\Delta\Delta\text{Ct}}$  method, and the gene expression in the control group was set as the standard in the analysis (Table 1).

## 2.6 Promotional and antagonistic effects of symbiotic strains on tobacco

### 2.6.1 Tobacco inoculation with growth-promoting and antagonistic bacteria

The test treatments were divided into four groups (Table 2): control group: only 20 ml of sterile water was applied; pathogenic group: the black shank pathogen was inoculated by stem inoculation to the susceptible tobacco variety Hongda as a pathogen infestation test group; probiotic group: the four probiotic strains were singly applied to the tobacco by root irrigation as a bacterial suspension; antagonistic group: the four probiotic strains were coinoculated with the pathogenic fungus as an antagonistic test.

### 2.6.2 Determination of agronomic traits and enzyme activity of tobacco

The natural plant height, crown width, leaf length and leaf width of the seedlings were measured with a straightedge (accuracy 0.1 cm); SOD, POD, PPO, PAL and enzyme activities were determined by a visible spectrophotometric method using a Solarbio kit (Purchased from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China).

### 2.6.3 Tobacco growth-related resistance gene expression

Following the above steps, RNA was extracted from tobacco plants and reverse-transcribed into cDNA, and finally, the expression of tobacco growth resistance-related genes was determined using the *NtActin* gene as an internal reference gene, as shown in Table 3. The ChamQ Universal SYBR qPCR Master Mix kit (Purchased from Vazyme Biotech Co., Ltd Nanjing, China). was selected for the validation of related enzymes and resistance-related gene expression levels. The

TABLE 1 Gene primers for quantitative real-time (qRT)-PCR.

Gene ID	Gene	Primer	Sequence
–	<i>Actin</i>	Forward	CAGCAGATGTGGATCTCAAAGG
		Reverse	GCGAACCAATCATAACCAAGCA
c12200.graph_c1	<i>FPPS</i>	Forward	GGGAGATGTTGGATTGATTAC
		Reverse	CAACCGGAAGGTAGAAGGAATAA
c102286.graph_c0	<i>SS</i>	Forward	ACCAAAGTCGCGGATGTCT
		Reverse	CCCCGTCGCATTTTGACAAC
c86496.graph_c0	<i>CAS</i>	Forward	CTCTTGGGCTGTTTGCTTAC
		Reverse	CCAACCACCAGAAGGCAACT
c165774.graph_c0	<i>GPPS</i>	Forward	AAGCTCATGGGGTTGGAGAG
		Reverse	ACTTTTGGCGGTAGGCAATG
c8027.graph_c0	<i>HMGS</i>	Forward	ACTCTGGCTGGTCAACGAGT
		Reverse	GAACAGTTGCAGGAAGAGC
–	<i>SE1</i>	Forward	GGACAGGCTGCTTCGATTA
		Reverse	TCAGTTGGCGGGATTCTTC
–	<i>SE2</i>	Forward	TGGCACCTCAGATTCCAAGT
		Reverse	CGGTCATTCTCCACCAGTTA

Actin is the reference gene; FPPS, SS, CAS, GGPS, HMGS, SE1, and SE2 are seven related expressed genes of the steroid saponin synthesis pathway. The primer sequences were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China).

TABLE 2 Test setup.

Processing	Repeat 1	Repeat 2	Repeat 3	Repeat 4	Repeat 5	Repeat 6
Control	CK	CK	CK	CK	CK	CK
Pathogenic group	YM	YM	YM	YM	YM	YM
Promotion group	TPD11	TPD11	TPD11	TPD11	TPD11	TPD11
	TPB	TPB	TPB	TPB	TPB	TPB
	LgD10	LgD10	LgD10	LgD10	LgD10	LgD10
	LgD2	LgD2	LgD2	LgD2	LgD2	LgD2
Antagonist group	YM+ TPD11	YM+ TPD11	YM+ TPD11	YM+ TPD11	YM+ TPD11	YM+ TPD11
	YM+ TPB	YM+ TPB	YM+ TPB	YM+ TPB	YM+ TPB	YM+ TPB
	YM+ LgD10	YM+ LgD10	YM+ LgD10	YM+ LgD10	YM+ LgD10	YM+ LgD10
	YM+ LgD2	YM+ LgD2	YM+ LgD2	YM+ LgD2	YM+ LgD2	YM+ LgD2

YM indicates the treatment of inoculated *Phytophthora nicotianae*; TPD11 and TPB were two symbiotic fungi, and LgD2 and LgD10 were two symbiotic bacteria.

PCR conditions were as follows: predenaturation phase 95°C for 30 s, cycling phase 95°C for 10 s, 60°C for 30 s, 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s, for a total of 40 cycles. After the reaction, the relative quantification of gene expression was performed using the  $2^{-\Delta\Delta C_t}$  method, and the gene expression in the control group was set as the standard in the analysis.

## 2.7 Data processing

The experimental data obtained were statistically analyzed by Excel 2013 software; SPSS 19.0 (SPSS, Chicago, IL, USA) analysis software was used for statistical analysis, and one-way analysis of variance (ANOVA) was used for the significance of

differences. Comparison of means between treatments was performed using Duncan's multiple comparison method for significance of differences, and Sigmaplot 10.0 software was used for graphing.

## 3 Results

### 3.1 Evaluation of the probiotic function of symbiotic strains

Symbiotic strains were isolated, 73 symbiotic strains were obtained from *P. fargesii* Franch, and the growth-promoting ability of the symbiotic strains was measured. The results

TABLE 3 Tobacco gene primers for qRT-PCR.

Gene ID	Gene	Primer	Sequence
107831145	<i>NtActin</i>	Forward	TGCCCGATGGTCAAGTTATC
		Reverse	GGATTCTGCAGCTTCCATTC
107805360	<i>NtPPO</i>	Forward	TGATAACTAATGCTCCTTGC
		Reverse	CTCCGAGTTCAACCAATCT
107830263	<i>NtSOD</i>	Forward	CCGTCGCCAAATTGCATAG
		Reverse	CGATAGCCCAACCAAGAGAAC
107796457	<i>NtHSR203J</i>	Forward	AGGAAGTATCCGGCTGGCTTAGA
		Reverse	GAAGTAGTCATGGGGTGGGACTG
107800718	<i>NtTTG2</i>	Forward	AGCGGAGTTGAAAGGCATCAGG
		Reverse	ATCAATCCCATTAGGCCAGCAA
107809964	<i>NbDADIR</i>	Forward	GTGTAGGCACTGCAGTCTTGCT
		Reverse	CTGCATAAGCACGTTCTGGTGGC
107822827	<i>NbRbohA</i>	Forward	ATCGTATCAGGCACCAGG
		Reverse	CGCCACAGTAGAAAACCC
107774415	<i>NbRbohB</i>	Forward	AACTTTAATACAAGGAGGGC
		Reverse	AAGAGCAGAACGAGCAT
107763263	<i>NtPR1a</i>	Forward	AACCTTTGACCTGGGACGAC
		Reverse	GCACATCCAACACGAACCGA
107792668	<i>PAL</i>	Forward	CGATAGACTTGAGGCATTGG
		Reverse	TCAGTGGGTAGTTGGCGATG

*NtActin* is the reference gene; *NtPPO*, *NtSOD*, *NtHSR203J*, *NtTTG2*, *NbDADIR*, *NbRbohA*, *NbRbohB*, *NtPR1a*, and *PAL* are tobacco disease resistance-related genes and enzyme-related genes. (Tobacco genetic database from: <https://www.ncbi.nlm.nih.gov/gene/>) The primer sequences were synthesized by Tsingke Biotechnology Co., Ltd. (Beijing, China).

showed that among the 43 symbiotic bacteria (Table 4), 25 strains had the ability to fix nitrogen, 5 strains had the ability to dissolve phosphorus, 22 strains had the ability to dissolve potassium, and 17 strains could produce IAA; among the 31 symbiotic fungi (Table 5), 4 strains had the ability to dissolve phosphorus, 31 strains had the ability to dissolve potassium, and 9 strains could produce IAA.

The 72 isolated symbiotic strains were cultured with *Phytophthora nicotianae* in plate standoffs, and the growth of the strains was observed after 7 d. Nine strains were found to have inhibitory effects on pathogenic fungus. Among these strains, LgD2, LgD10, TPB and TPD11 had the strongest inhibitory effect; therefore, these four strains were cultured in a secondary standoff with the pathogen, and the diameter of the control and treated pathogens was measured after 7 d. As shown in Table 6, the two symbiotic bacterial strains LgD2 and LgD10 showed 90 and 88% inhibition against *Phytophthora nicotianae*, while the two symbiotic fungal strains TPB and TPD11 showed 92 and 81% inhibition against *Phytophthora nicotianae*.

### 3.2 Phylogenetic analysis of antagonistic probiotic strains

Four strains (LgD2, LgD10, TPB and TPD11) were selected for subsequent growth promotion tests based on their growth promotion characteristics and their ability to antagonize

pathogenic fungi. The 16S rDNA and ITS sequences of the four selected strains of *P. fargesii* Franch. were compared with multiple sequences using the software ClustalX2, and then the software MEGA4 was used to construct a phylogenetic tree according to the neighbor-joining method, as shown in Figures 1 and 2. Two strains of the interrhizosphere fungus TPD11 clustered with *Aspergillus tamarii* (OP405014), and TPB clustered with *Fusarium oxysporum* (OP405007); two strains of the endophytic bacterium LgD10 clustered with *Bacillus thuringiensis* (OP404360), and strain LgD2 clustered with *Bacillus cereus* (OP404241). The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank (<https://www.ncbi.nlm.nih.gov/>)

### 3.3 Symbiotic culture of growth-promoting strains and *Pairs polyphylla* var. *yunnanensis* seedlings

#### 3.3.1 The effect of probiotic strains on the biomass of *P. polyphylla* var. *yunnanensis*

After the above screening, four symbiotic strains (LgD2, LgD10, TPB and TPD11) were cultured in symbiosis with *P. polyphylla* var. *yunnanensis* seedlings, the external morphology of the seedlings after coculture was observed, and the seedlings grew well between treatments without disease infection traits

TABLE 4 Analysis of the growth-promoting potential of symbiotic bacteria.

No.	Phosphorus solubilization	Potassium solubilization	Nitrogen fixation	IAA production
LgA2	+	+	+	+
LgA3	-	+	+	+
LgA5	-	+	+	+
LgA6	-	+	+	+
LgA8	-	+	++	+
LgA16	-	+	+	+
LgA17	-	+	+	+
LgB4	-	-	-	-
LgC2	-	-	+	-
LgC3	-	-	-	-
LgC9	-	-	-	-
LgD2	+	++	++	+++
LgD9	-	-	-	-
LgD10	+	++	++	+++
LgD11	-	+	+	+
Lgf2	-	+	+	+
Lgf3	-	-	-	-
Lgf4	-	-	+	-
Lgf6	-	-	-	-
Lgf7	-	+	+	+
Lgf8	-	+	+	+
TL3	-	-	-	-
TL4	-	+	+	++
TL5	-	-	-	-
TL6	-	+	+	+
TL8	-	-	-	-
TL9	-	+	+	+
TLB12	-	-	+	-
TLB13	-	-	+	-
TLB14	+	+	+	++
TLC7	-	-	-	-
TLC11	-	-	-	-
TLD6	-	+	+	-
TLD5	+	+	-	-
TLD2	-	+	-	-
TLE1	-	+	+	-
TLD3	-	-	-	-
TLC5	-	-	-	-
TLD10	-	-	-	-
TLB7	-	-	+	-
TLE3	-	-	-	-
TLE8	-	-	-	-
TLE2	-	+	+	-

According to the growth of different strains on the plate, the nitrogen fixation ability of the strains was divided into three grades: high, medium and low, which were marked as +, ++, and +, respectively. The strains that did not grow on the plate were labeled as having no nitrogen fixation ability, which was marked as -.

(Figure 3). Some of the agronomic traits of *P. polyphylla* var. *yunnanensis* were evaluated (Table 7). The four inoculated symbiotic strains of TPB, LgD10, TPD11 and LgD2 treatments were all significantly taller than the control CK, at 55, 47, 44 and 43% higher than the control, respectively. The five treatments in descending order of growth in plant height were TPB > LgD10 > TPD11 > LgD2 > CK. The greatest effect on plant height was found in the TPB strain, followed by LgD10, while the difference between TPD11 and LgD2 was not significant. The treatments inoculated with the TPB strain had significantly higher fresh weight of the aboveground parts, leaf length and leaf width than the control by 65, 46 and 42%, respectively; treatments inoculated with the TPD11 strain by 53, 46 and 41%, respectively; the treatments inoculated with the LgD10 strain were 52.38, 42.19 and 38% higher, respectively; and the treatments inoculated with the LgD2 strain were 48, 43 and 37% higher, respectively. The main medicinal part of *P. polyphylla* var. *yunnanensis* is the rhizome, so the root weight and root length of its underground part were also measured separately. The results showed that the root weight and root length of the underground parts of the treatments inoculated with the growth-promoting bacteria and fungi were significantly higher than the root weight and root length of the underground parts of the control. The ground weight of the treatment inoculated with the TPB strain was 46% higher than the ground weight of the control, while TPD11, LgD2 and LgD10 were not significantly different. The longest roots were found in the treatment inoculated with the TPB strain, which was 50% longer than the control, followed by the LgD2 strain treatment, which was 42% longer than the control, while the difference between TPD11 and LgD10 was not significant. In summary, all four symbiotic strains increased the plant height, leaf length and leaf width of *P. polyphylla* var. *yunnanensis* promoted the fresh weight of the above- and belowground parts of *P. polyphylla* var. *yunnanensis* and stimulate root elongation and promote the development of the root system of *P. polyphylla* var. *yunnanensis*.

### 3.3.2 Effect of probiotic strains on the chloroplast pigment content of *P. polyphylla* var. *yunnanensis*

As shown in Figure 4, the chlorophyll a, chlorophyll b and total chlorophyll content of *P. polyphylla* var. *yunnanensis* seedlings inoculated with symbiotic strains were significantly higher than the chlorophyll a, chlorophyll b and total chlorophyll content of the control. With a peak at the LgD10 strain treatment, the values were 0.31, 0.57 and 0.89 (mg g<sup>-1</sup> fresh weight (FW)), respectively, followed by the LgD2 strain treatments at 0.31, 0.56 and 0.86 (mg g<sup>-1</sup> FW), respectively. The TPB strain treatments contained 0.26, 0.55 and 0.82 (mg g<sup>-1</sup> FW),



TABLE 5 Analysis of the growth-promoting potential of the symbiotic fungus.

No.	Dissolved phosphorus	Potassium dissolution	Production IAA
TP0	-	+	-
TPB	+	+	+
TP11	-	+	+
TP13	-	+	-
TP14	-	+	+
TP2	-	+	-
TP6	-	+	+
TP8	+	+	+
TPC1	-	+	+
TPC5	-	+	-
TPC6	-	+	-
TPC8	-	+	-
TPD	-	+	+
TPD10	-	+	-
TPD6	-	+	-
TPD7	-	+	-
TPD8	-	+	-
TPE3	-	+	-
TPD11	+	+	+
pgG1	+	+	+
A8	-	+	-
G4	-	+	-
A5	-	+	-
A6	-	+	-
A2	-	+	-
A4	-	+	-
C4	-	+	-
D2	-	+	-
TP10	-	+	-
TPE4	-	+	-
TPE5	-	+	-

According to the growth of different strains on the plate, the nitrogen fixation ability of the strains was divided into three grades: high, medium and low, which were marked as +, ++, and +, respectively. The strains that did not grow on the plate were labeled as having no nitrogen fixation ability, which was marked as -.

TABLE 6 Inhibitory effect of different strains on pathogens.

No.	Control colony radius (cm)	Radius of treated colonies (cm)	Bacterial inhibition rate (%)
CK	6.5	0	0
TPD11	6.5	5.3	81
TPB	6.5	6	92
LgD10	6.5	5.8	90
LgD2	6.5	5.7	88

CK was the only pathogen of *Phytophthora nicotianae*; TPD11 and TPB were two symbiotic fungi, and LgD2 and LgD10 were two symbiotic bacteria.

respectively. TPD11 strain treatments contained 0.24, 0.54 and 0.79 (mg g<sup>-1</sup> FW), respectively. The chlorophyll a, chlorophyll b and total chlorophyll content among the five treatments decreased in the order LgD10 > LgD2 > TPB > TPD11 > CK. The chlorophyll a/b was significantly higher in the treatment CK without inoculation of probiotic bacteria and fungi than in the other treatments, while the differences between the treatments of LgD10 and LgD2 strains were not significant.

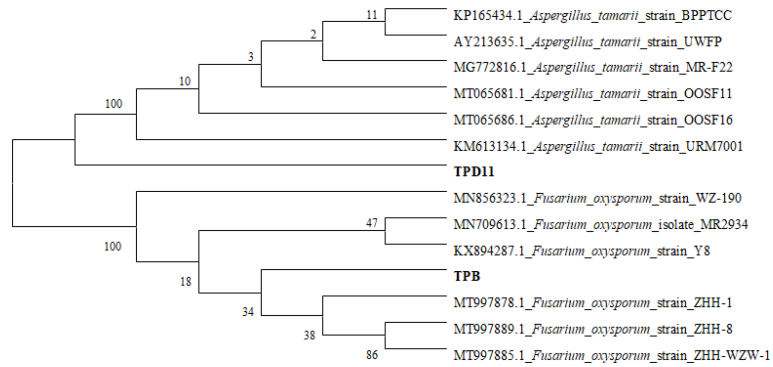
### 3.3.3 Effect of probiotic strains on the gas exchange parameters of *P. polyphylla* var. *yunnanensis*

After the coculture of four strains of symbiotic strains with two-year-old *P. polyphylla* var. *yunnanensis* seedlings, the gas exchange parameters of the plants were measured for each treatment and statistically analyzed, and the results are shown in Table 8. Inoculation with four strains of probiotic bacteria and fungi significantly increased the net photosynthetic rate (Pn), stomatal conductance (Cond), transpiration rate (Tr) and intercellular CO<sub>2</sub> concentration (Ci) of *P. polyphylla* var. *yunnanensis* leaves compared with CK. Statistically, the treatment of the TPD11 strain had the highest net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO<sub>2</sub> concentration among all treatments, followed by the TPB, LgD10 and LgD2 strain treatments. Overall, inoculation with probiotic bacteria enhanced photosynthesis in the leaves of *P. polyphylla* var. *yunnanensis*.

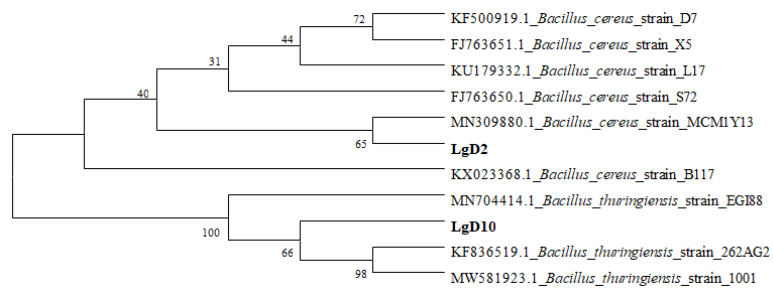
### 3.3.4 Effect of probiotic bacteria and fungi on the saponin content of *P. polyphylla* var. *yunnanensis*

After single back inoculation with four strains of symbiotic strains, the contents of Parissaponin I, Parissaponin II, Parissaponin VI, Parissaponin VII, Parissaponin D, Parissaponin H and Rhizoma Paridis total saponins were determined in the treated rhizomes of *P. polyphylla* var. *yunnanensis*. The contents were calculated against Parissaponin standards, and the statistical results are shown in Figure 5. There was a significant effect on the yield of Parissaponin after a single back-injection of the symbiotic strains.

Parissaponin I had the highest content after treatment with the LgD2 strain at 0.049%, followed by LgD10, TPD11 and TPB at 0.034, 0.038 and 0.019%, respectively. The Parissaponin I content in the LgD2 strain treatment was significantly higher than the Parissaponin I content in the LgD10, TPD11 and TPB strains; there was no significant difference between the LgD10 and TPD11 strain treatments, and Parissaponin I was not detected in the CK treatment. The inoculation of four strains of LgD2, LgD10, TPD11 and TPB treated with Parissaponin II decreased in the order of LgD2 > LgD10 > TPD11 > TPB > CK, with values of 0.613, 0.422, 0.264, 0.155 and 0.056%, respectively. The content of Parissaponin VI was highest under the LgD10



**FIGURE 1**  
Phylogenetic analysis of the TPB and TPD11 strains. ITS regions of fungal isolates, with the sequences from NCBI TPD11 and TPB being the sequences obtained from fungi. Phylogenetic trees were constructed by the neighbor-joining method using the software MEGA4.



**FIGURE 2**  
Phylogenetic analysis of the LgD2 and LgD10 strains. 16S rRNA sequences of the bacterial isolates, with the sequences from NCBI. LgD2 and LgD10 refer to 16S rRNA sequences retrieved from bacteria. Phylogenetic trees were constructed by the neighbor-joining method using the software MEGA4.

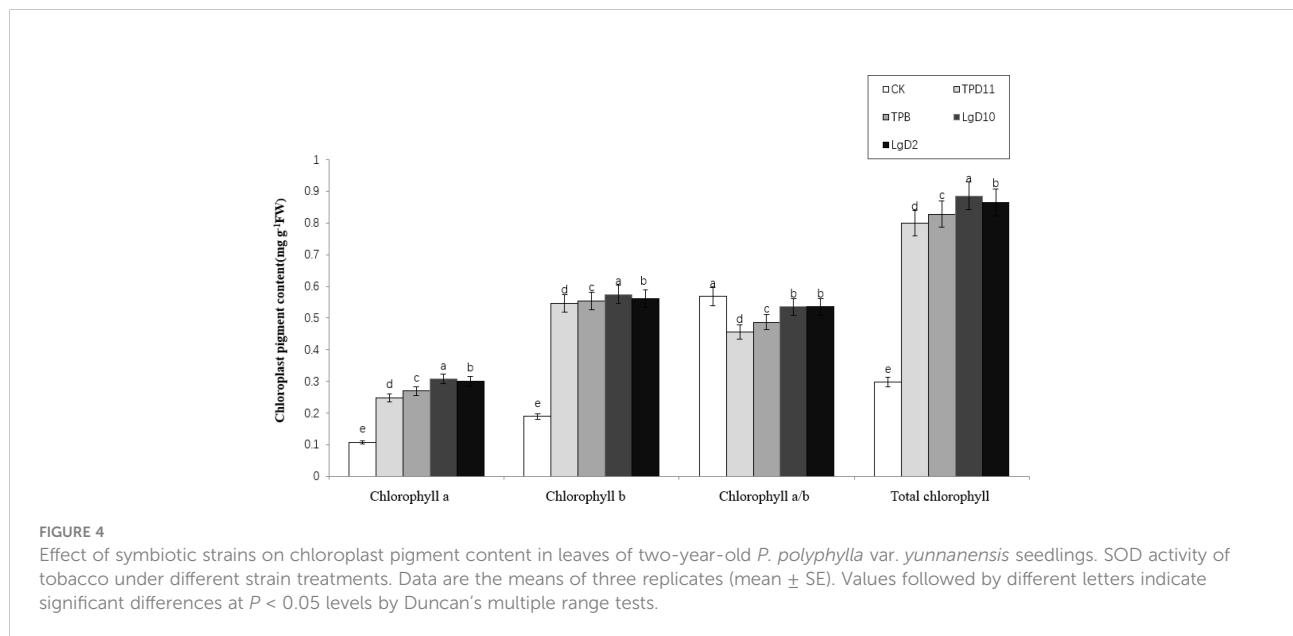


**FIGURE 3**  
Two-year-old *P. polyphylla* var. *yunnanensis* seedling morphology after symbiotic strain treatment. CK is distilled water treatment; TPD11 and TPB were two symbiotic fungal treatments, and LgD2 and LgD10 were two symbiotic bacterial treatments.

TABLE 7 Effects of symbiosis on the biomass of two-year-old *P. polyphylla* var. *yunnanensis* seedlings.

Processing	Plant height cm	Root length cm	Root weight g	Leaf length cm	Leaf width cm	Fresh weight on the ground g
CK	9.7 ± 0.25d	6.26 ± 0.14d	0.53 ± 0.02d	2.00 ± 0.05c	1.26 ± 0.03d	0.30 ± 0.01c
TPD11	17.17 ± 0.14c	9.00 ± 0.41c	0.77 ± 0.01bc	3.70 ± 0.05a	2.13 ± 0.03ab	0.64 ± 0.02b
TPB	21.67 ± 0.33a	12.50 ± 0.28a	0.98 ± 0.02a	3.73 ± 0.03a	2.20 ± 0.00a	0.87 ± 0.02a
LgD10	18.33 ± 0.33b	8.96 ± 0.08c	0.82 ± 0.01b	3.46 ± 0.03b	2.03 ± 0.03bc	0.63 ± 0.01b
LgD2	17.10 ± 0.21c	10.80 ± 0.11b	0.72 ± 0.01c	3.53 ± 0.03b	2.00 ± 0.00c	0.58 ± 0.01b

Data are the mean ± standard error (n=3, 3 biological replicates), and data were tested for differences using Duncan's method, with different letters representing highly significant differences (P<0.05). TPD11 and TPB are two symbiotic fungi, and LgD2 and LgD10 are two symbiotic bacteria.



strain, and the content after inoculation treatment was LgD10 > LgD2 > TPB > TPD11 > CK in descending order, 0.071, 0.039, 0.032, 0.031 and 0.016%, respectively. The content of LgD2 was significantly higher than the content of the TPD11 and TPB strain treatments, while the TPD11 and TPB strain treatments were not significantly different from each other. The content of Parissaponin VII in the inoculated probiotic treatment was significantly higher than the Parissaponin VII content in the control, from high to low, LGD10 > LGD2 > TPD11 > CK > TPB, with values of 0.396, 0.347, 0.208, 0.149 and 0.142%, respectively. Parissaponin D was detected only under the CK and TPB strain treatments at 0.022 and 0.016%, respectively, while not being detected in the rest of the treatments. Parissaponin D was detected only under the CK and TPB strain treatments at 0.022 and 0.016%, respectively, while Parissaponin D was not detected in the rest of the treatments. The *P. polyphylla* var. *yunnanensis* total saponin treatments inoculated with probiotic bacteria and fungi alone were significantly higher than the control, from high to low, LgD2 > LgD10 > TPD11 > TPB > CK, 1.049, 0.951, 0.563, 0.387 and

0.262%, respectively. In brief, all four strain treatments increased the rhizome Parissaponin content of seedlings to varying degrees, with particularly pronounced changes in the total saponin content of *P. polyphylla* var. *yunnanensis*.

### 3.3.5 Expression analysis of genes of the Parissaponin synthesis pathway induced by probiotic bacteria and fungi

To investigate the effect of symbiotic strains on the expression of genes related to the saponin synthesis pathway of *P. polyphylla* var. *yunnanensis*, quantitative fluorescence PCR analysis of key genes of the steroidal saponin synthesis pathway (*HMGS*, *GGPS*, *FPPS*, *SS*, *SE1*, *CAS*, *SE2*), which were previously reported by previous authors, was performed, and *Actin* was used as an internal reference gene to calculate the expression results, as shown in Figure 6. All treatments differentially increased the expression of Parissaponin synthesis pathway genes, with the highest expression of *HMGS*, *SE2*, and *CAS* under the LgD10 strain treatment, the highest expression of *GGPS* and *FPPS* under the TPD11 strain treatment, and the

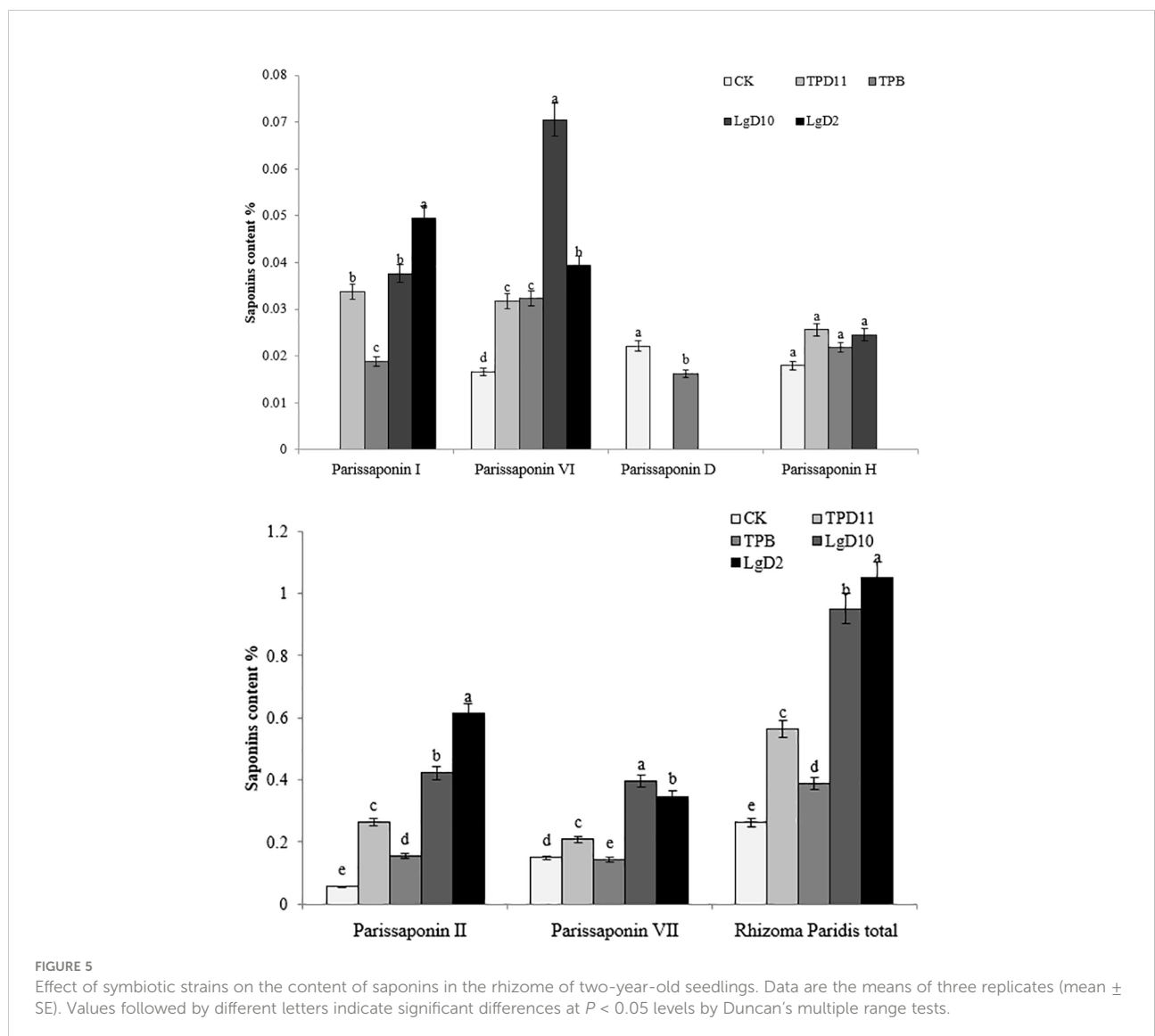
TABLE 8 Effect of symbiotic strains on gas exchange parameters in leaves of two-year-old seedlings.

Processing	Pn( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Cond( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Ci( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Tr( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )
CK	2.73 ± 0.01e	0.053 ± 0.00e	297.11 ± 0.04e	0.88 ± 0.00c
TPD11	4.51 ± 0.00a	0.11 ± 0.00a	320.77 ± 0.03a	1.33 ± 0.00a
TPB	3.64 ± 0.02b	0.08 ± 0.00b	318.26 ± 0.22b	0.86 ± 0.00d
LgD10	3.45 ± 0.01c	0.07 ± 0.00c	310.73 ± 0.33c	0.92 ± 0.00b
LgD2	3.30 ± 0.00d	0.06 ± 0.00d	308.11 ± 0.05d	0.82 ± 0.00e

Data are the mean ± standard error (n=3, 3 biological replicates), and data were tested for differences using Duncan’s method, with different letters representing highly significant differences (P<0.05); TPD11 and TPB were two symbiotic fungi, and LgD2 and LgD10 were two symbiotic bacteria.

highest expression of *SE1* under the LgD2 treatment. The expression of *HMGS* was upregulated in the treatments with four strains of probiotic bacteria and fungi TPB, LgD10, TPD11 and LgD2, which were 0.3, 1.9, 2.5 and 2.2 times higher than the control, respectively, and the difference between TPD11 and the

control was not significant; the expression of the *GGPS* gene was significantly upregulated in the four strains of probiotic bacteria and fungi inoculated separately, which were 7.8, 1.6, 4.6 and 3.1 times higher than the control, respectively. The expression of the *FPPS* gene was upregulated in all four strains, 10.1, 1.4, 8.3 and



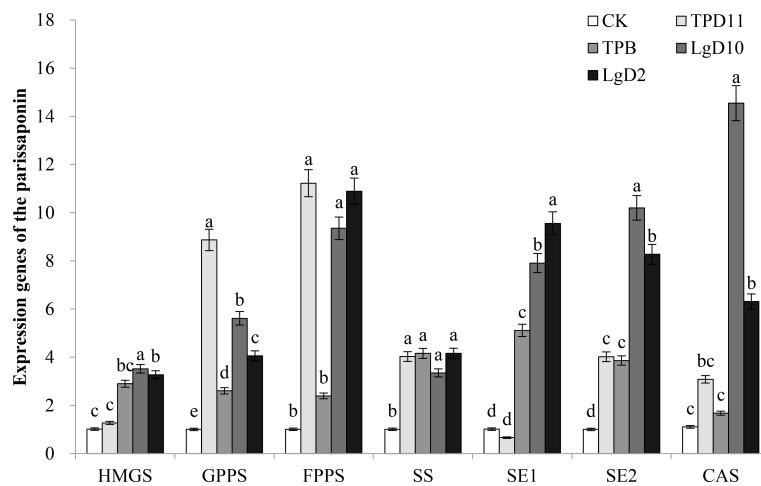


FIGURE 6

Effect of symbiotic strains on the expression of related genes in the rhizome of two-year-old seedlings. Data are the means of three replicates (mean  $\pm$  SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

9.8 times that of the control, respectively, and the difference was not significant among TPD11, LgD10 and LgD2. TPB was upregulated but not significantly different from the control. The expression of the *SS* gene was significantly upregulated in all four strains, 3.0, 3.1, 2.3 and 3.1 times that of the control, respectively, and the difference was not significant among the four strains. The expression of the *SE1* gene was significantly upregulated in all four strains, TPD11, LgD10 and LgD2. The expression of the *SE1* gene was significantly upregulated by inoculation with the TPD11, LgD10 and LgD2 strains, which was 4.0, 6.8 and 8.4 times higher than the expression of the *SE1* gene of the control, respectively, and treatment with the TPB strain suppressed the expression of the *SE1* gene. The expression of the *SE2* gene was upregulated by inoculation of the four strains of probiotic treatment, 3.0, 2.9, 9.2 and 7.3 times more than the control, respectively, and the difference between TPB

and TPD11 was not significant; the expression of the *CAS* gene was upregulated by inoculation of the four strains of probiotic treatment, 1.8, 0.5, 12.1 and 4.7 times more than the control, respectively, and the difference between TPB and TPD11 was not significant compared with the control.

### 3.4 Effect of inoculation of tobacco with probiotic and pathogenic fungi on tobacco physiology

#### 3.4.1 Effect of growth-promoting bacteria and fungi on tobacco biomass

Tobacco biomass was significantly higher in the promotion group treatment as well as in the antagonist group treatment than in the control and pathogenic groups (Table 9). In the growth-

TABLE 9 Effects of growth-promoting antagonistic strains on tobacco growth.

Processing	Plant height cm	Crown size cm	Leaf length cm	Leaf width cm
CK	39.00 $\pm$ 0.57d	74.00 $\pm$ 0.57d	34.00 $\pm$ 1.00e	18.00 $\pm$ 1.00b
TPD11	46.33 $\pm$ 1.33b	78.33 $\pm$ 0.66bc	42.67 $\pm$ 1.20c	21.67 $\pm$ 0.88a
TPB	42.33 $\pm$ 2.02cd	80.66 $\pm$ 1.20ab	44.33 $\pm$ 1.20bc	22.67 $\pm$ 0.66a
LgD10	48.33 $\pm$ 1.76ab	82.33 $\pm$ 2.02a	46.67 $\pm$ 1.76ab	23.67 $\pm$ 0.66a
LgD2	49.00 $\pm$ 1.15a	81.00 $\pm$ 0.57ab	45.00 $\pm$ 1.15abc	22.67 $\pm$ 0.88a
YM	41.67 $\pm$ 1.20cd	62.67 $\pm$ 1.20e	39.00 $\pm$ 0.27d	19.33 $\pm$ 0.33b
TPD11+Y	45.00 $\pm$ 0.57bc	77.67 $\pm$ 0.33c	45.00 $\pm$ 0.57abc	23.00 $\pm$ 0.57a
TPB+Y	46.67 $\pm$ 0.33ab	76.00 $\pm$ 0.57cd	46.00 $\pm$ 0.00ab	22.00 $\pm$ 0.57a
LgD10+Y	47.33 $\pm$ 0.33ab	75.67 $\pm$ 0.33cd	48.00 $\pm$ 1.00a	24.00 $\pm$ 0.57a
LgD2+Y	47.00 $\pm$ 1.00ab	75.33 $\pm$ 0.33cd	44.67 $\pm$ 0.33bc	22.00 $\pm$ 0.57a

The data in the table are the mean  $\pm$  standard error ( $n = 3$ ). Different lowercase letters after the same data show that the differences were significant ( $P < 0.05$ ); TPD11 and TPB were two symbiotic fungi, and LgD2 and LgD10 were two symbiotic bacteria.

promoting group, plant height, crown width, leaf length and leaf width increased by 20, 8, 24 and 21% in the LgD2 strain treatment; 19, 10, 27 and 24% in the LgD10 strain treatment; and 16, 6, 20 and 17% in the TPD11 strain treatment, respectively, compared to the control. TPB strain treatments increased by 8, 8, 23 and 21%, respectively. Compared to CK, the LgD2 and LgD10 strain treatments had the greatest effect on tobacco plant height, crown width and leaf length, and the differences between them were not significant. The height, crown width, leaf length and leaf width of tobacco plants treated with *Phytophthora nicotianae* alone were significantly lower than the height, crown width, leaf length and leaf width of the antagonistic group treated with the four probiotic strains and *Phytophthora nicotianae* together, where LgD2+Y increased by 11, 17, 13 and 12%, respectively, compared to YM inoculation alone; LgD10+Y increased 12, 17, 19, and 19%, respectively, over YM inoculation alone; TPB+Y improved 11, 18, 15, and 12%, respectively, compared with YM inoculation alone; and TPD11+Y improved 7, 19, 13, and 16%, respectively, compared with YM inoculation alone. The differences in plant height and leaf width in the pathogenic group treatments were not significant compared to the control.

### 3.4.2 Effect of probiotic bacteria and fungi on the enzyme activity of tobacco

#### 3.4.2.1 Effect of probiotic bacteria and fungi on SOD enzyme activity of tobacco

The SOD enzyme activities of the probiotic antagonist strains, pathogenic fungus and tobacco were measured at the end of coculture, and the results are shown in Figure 7. The SOD enzyme activity of the treatments in the promotion group inoculated with three strains of the probiotic antagonist strains, TPD11, LgD10 and LgD2, alone were higher than the control CK by 5, 24 and 4%, respectively, but the difference

between LgD2 and the control was not significant, while the SOD enzyme activity of the TPB inoculation treatment was 18% lower than the control. All three treatments, TPD11+Y, LgD10+Y and LgD2+Y, in the antagonistic group had significantly higher SOD enzyme activity than the pathogenic group treatment YM, 8, 6 and 3% higher than YM, respectively, and the differences among the three probiotic group treatments were not significant. The enzyme activity of the TPB+Y inoculation treatment was 18% lower than the enzyme activity of the control. The SOD enzyme activity of YM inoculated with *Phytophthora nicotianae* alone was significantly lower than the SOD enzyme activity of the control CK.

#### 3.4.2.2 Effect of probiotic bacteria and fungi on POD enzyme activity of tobacco

The POD enzyme activities of the probiotic antagonist strain, pathogenic fungus and tobacco were measured at the end of coculture, and the results are shown in Figure 8. The POD enzyme activities of TPD11, TPB, LgD10 and LgD2 were significantly higher than the control CK in the promotion group treatment and 18, 7, 32 and 42% higher than the control, respectively, and the POD enzyme activities of the four strains of promotion antagonists decreased in the order LgD2> LgD10> TPD11> TPB. The POD enzyme activity of TPD11+Y, TPB+Y, LgD10+Y and LgD2+Y in the antagonist group treatment was significantly higher than the POD enzyme activity of the pathogenic group treatment YM, 13, 8, 26 and 21% higher than the POD enzyme activity of YM, respectively, and the enzyme activities were ranked as LgD10+Y>LgD2+Y>TPD11+Y>TPB+Y, respectively. The POD enzyme activity of YM treated with *Phytophthora nicotianae* alone was significantly higher than the POD enzyme activity of the control CK.

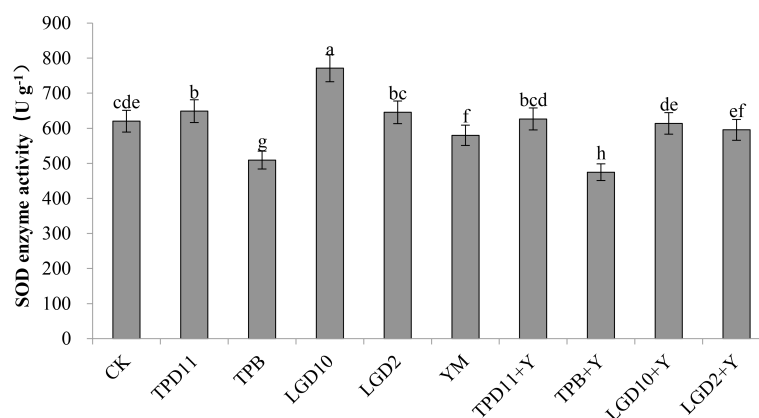
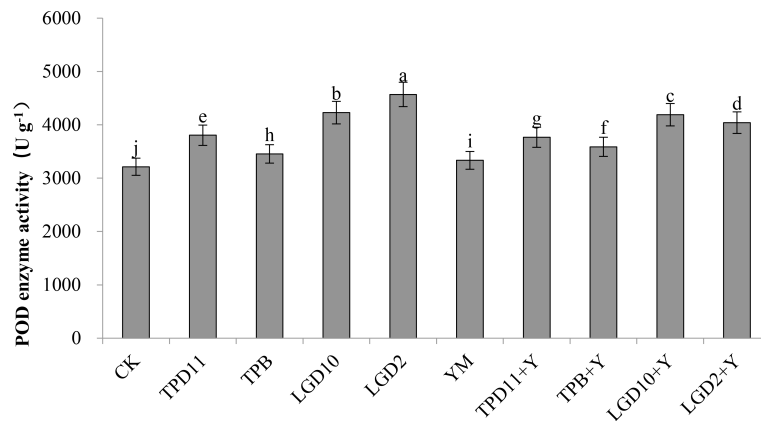


FIGURE 7

SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean  $\pm$  SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.



**FIGURE 8**  
POD activity of tobacco under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

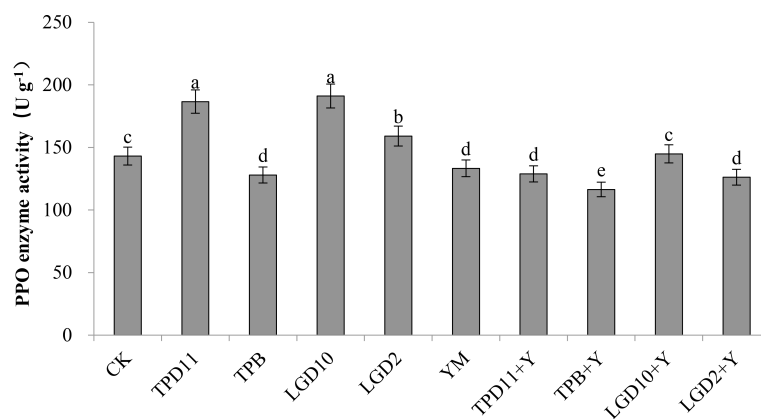
### 3.4.2.3 Effect of probiotic bacteria and fungi on PPO enzyme activity in tobacco

The PPO enzyme activity of the probiotic antagonist strain, pathogenic fungus and tobacco was measured at the end of coculture, and the results are shown in Figure 9. The PPO activity of TPD11, LgD10 and LgD2 was significantly higher than the control CK in the promotion group treatment and 30, 34 and 11% higher than the control, respectively, and the PPO activity of the three strains of promotion antagonists was LgD10 > TPD11 > LgD2 in descending order, while the PPO activity of the TPB inoculation treatment were 11% lower than the control. Among the antagonist group treatments, only inoculation with LgD10+Y treatment PPO activity was significantly higher than YM treatment by 9%, while the

TPD11+Y and LgD2+Y treatments were not significantly different from YM treatment, and inoculation with TPB+Y treatment PPO activity was significantly lower than YM treatment by 13%. The PPO enzyme activity of YM treated with *Phytophthora nicotianae* alone was significantly lower than the PPO enzyme activity of the control CK.

### 3.4.2.4 Effect of probiotic bacteria and fungi on PAL enzyme activity in tobacco

The PAL enzyme activity of the probiotic antagonist strains, pathogenic fungus and tobacco were measured at the end of coculture, and the results are shown in Figure 10. The PAL activities of TPD11 and LgD10 were significantly higher than the control CK by 12 and 13% in the promotion group treatment,



**FIGURE 9**  
PPO activity of tobacco under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

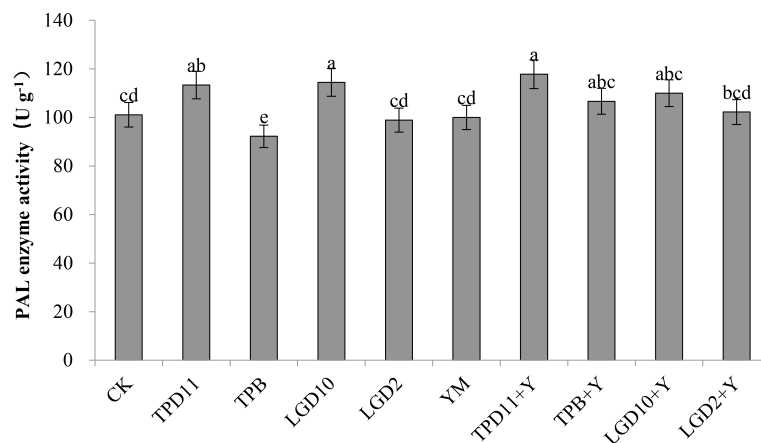


FIGURE 10

PAL activity of tobacco under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean  $\pm$  SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

while the difference between the PAL activity of the LgD2 inoculation treatment and the control was not significant, and the PAL activity of the TPB inoculation treatment were significantly lower than the control CK by 9%. The PAL activity of the four strains of promotion antagonists were, in descending order, LgD10 > TPD11 > LgD2 > TPB. The PAL enzyme activity of TPD11+Y, TPB+Y, LgD10+Y and LgD2+Y was higher in the antagonist group treatment than in the pathogenic group treatment YM, 18, 7, 10 and 2% higher than the PAL enzyme activity of YM, respectively, and only TPD11+Y enzyme activity was significantly higher than the PAL enzyme activity of the *Phytophthora nicotianae* treatment YM, while TPB+Y, LgD10+Y and LgD2+Y treatments were not significantly different compared to the YM treatment. The PAL activity of YM treated with *Phytophthora nicotianae* alone was not significantly different compared to the control CK.

### 3.5 Inhibition of tobacco pathogens by probiotic bacteria and fungi

After cocultivation of the antagonistic strain with tobacco, the number of diseased strains and disease levels were measured separately, and according to the formula for tobacco disease, incidence (%) = number of diseased strains/total number of plants investigated  $\times$  100%, disease index (%) =  $\sum$  (number of diseased strains at each level  $\times$  corresponding level)/total number of plants investigated  $\times$  highest level value  $\times$  100%, relative suppression (%) = (1 - disease index in treatment area/disease index in control area)  $\times$  100%, the incidence, disease index and relative suppression rate were calculated for each treatment, and the results are shown in Figure 11. The incidence of YM treatment alone reached 100%, and compared to YM alone,

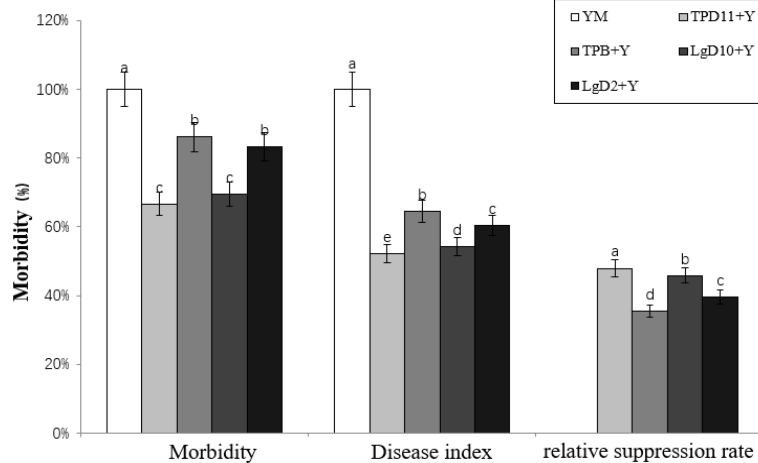
the incidence of TPD11+Y, TPB+Y, LgD10+Y, and LgD2+Y in the antagonist group treatment was reduced by 33, 14, 31, and 17%, respectively, and the incidence of TPD11+Y and LgD10+Y inoculation was significantly lower than that of the TPB+Y and LgD2+Y treatments. The disease indices of the TPD11+Y, TPB+Y, LgD10+Y and LgD2+Y treatments were significantly reduced, 48, 35, 46 and 40%, respectively, compared with the disease indices of the control, and the disease indices of the TPD11+Y inoculation treatments were significantly lower than the disease indices of the other treatments. Compared with the control, the relative inhibition rates of inoculation with TPD11+Y, TPB+Y, LgD10+Y and LgD2+Y treatments were significantly lower, 48, 35, 46 and 40%, respectively, and the lowest relative inhibition rate was reached under inoculation with TPD11+Y treatment.

### 3.6 Expression of defense enzymes and resistance-related genes by probiotic bacteria and fungi

#### 3.6.1 Effect of probiotic antagonistic bacteria and fungi on defense enzyme-related genes

PPO, PAL, SOD and POD are all enzymes that play a role in disease resistance in tobacco, and the *NtPPO*, *PAL*, *NtSOD* and *NbDADIR* genes control the synthesis of these four enzymes. As shown in Figure 12, *NtPPO* gene expression was significantly upregulated in the TPD11 and TPB+Y inoculations compared to the control, 92% and 73% higher than the control, followed by both LgD10 and LgD2+Y treatments, 73% and 69% higher than the control, respectively. Compared to the control, *PAL* genes were upregulated in TPD11- and LgD10-treated plants, 94 and 58% higher than the control, respectively, while the remaining



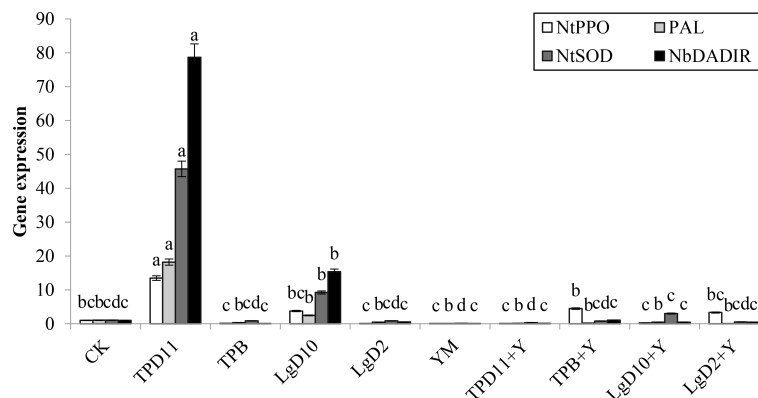


**FIGURE 11** Incidence of tobacco after inoculation with symbiotic strains. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

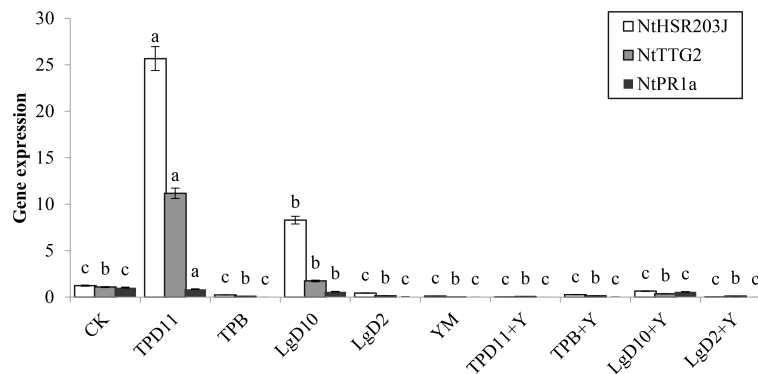
strains treated with *PAL* genes were not significantly different compared to the control. Plants inoculated with TPD11, LgD10 and LgD10+Y treatments had significantly upregulated *NtSOD* genes compared to the control, 99% and 65% higher than the control, respectively, while the *NtSOD* genes were not expressed in the plants of the remaining treatments. The expression of *NbDADIR* genes was upregulated in the inoculated TPD11, LgD10 and TPB+Y treatments, which were 99, 93 and 3% higher than the control, respectively, while the *NbDADIR* genes were not expressed in the plants of the remaining treatments. The expression of four enzyme genes, *NtPPO*, *PAL*, *NtSOD* and *NbDADIR*, peaked in tobacco leaves after inoculation with the TPD11 strain.

### 3.6.2 The expression of the tobacco growth-related resistance genes

*NtHSR203J*, *NtTTG2* and *NtPR1a* are related to the growth and disease resistance of tobacco. As shown in Figure 13, the expression of the *NtHSR203J* gene was significantly upregulated by the TPD11 and LgD10 treatments and was 95 and 85% higher than the expression of the *NtHSR203J* gene of the control, respectively, and the expression level of TPD11 was the highest. None of the other strains expressed these genes. The expression of the *NtTTG2* gene was upregulated in the inoculated TPD11 and LgD10 treatments, which was 90 and 38% higher than the expression of the *NtTTG2* gene in the control, respectively, and the *NtTTG2* gene was not expressed in the remaining strain



**FIGURE 12** Gene expression under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.



**FIGURE 13** Gene expression under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

treatments, which is consistent with the results of *NtHSR203J* gene expression. The *NtPR1a* gene was not expressed in all strain inoculation treatments.

### 3.6.3 Expression of resistance genes associated with black shank disease in tobacco

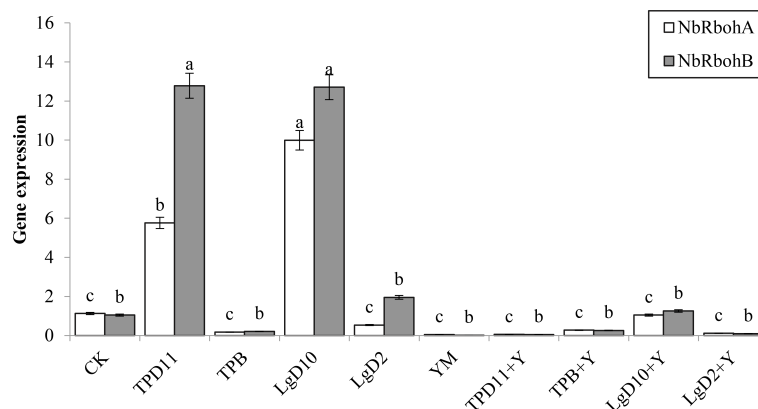
Tobacco *NbRbohA* and *NbRbohB* genes have important roles in tobacco resistance to *Phytophthora infestans*. As shown in Figure 14, the expression of the *NbRbohA* gene was upregulated in both the TPD11 and LgD10 treatments compared to the control, 80 and 89% higher than the control, respectively, and the expression of the *NbRbohA* gene in the LgD10+Y treatment also showed upregulation of the *NbRbohA* gene, but the difference was not significant, and the *NbRbohA* gene was not expressed in the rest of the treated tobacco plants. The expression of the *NbRbohB* gene was significantly upregulated

in both the TPD11 and LgD10 treatments and was 92 and 92% higher than the expression of the *NbRbohB* gene in the control, respectively. The *NbRbohB* gene was upregulated in both the LgD2 and LgD10+Y treatments, but the difference was not significant, and the *NbRbohA* gene was not expressed in the rest of the treated tobacco plants.

## 4 Discussion

### 4.1 Functional studies on the probiotic antagonism of symbiotic strains

In this study, we isolated 73 symbiotic strains from the rhizomes and interrhizosphere soil of bacteria isolated from *P. fargesii* Franch. By screening all the symbiotic strains for their



**FIGURE 14** Gene expression under different strain treatments. SOD activity of tobacco under different strain treatments. Data are the means of three replicates (mean ± SE). Values followed by different letters indicate significant differences at  $P < 0.05$  levels by Duncan's multiple range tests.

pro- and antagonistic effects, we aimed to screen the strains that showed the most promising applications in promoting the growth of *P. polyphylla* var. *yunnanensis* and the accumulation of the medicinal active ingredient saponin and controlled tobacco black shank. Symbiotic strains provide nitrogen, phosphorus and potassium nutrients to plants through biological nitrogen fixation, dissolved phosphate and solid potassium (Banerjee, 2005; Zhao et al., 2009; Nag et al., 2020), promote the absorption of nutrients by the host plant and provide the vitamins needed for plant growth. Symbiotic strains can increase the root surface area and length by producing IAA to stimulate plant cell elongation or affect cell division, thereby increasing plant access to more soil nutrients and directly promoting plant growth (Woodward and Bartel, 2005). The most powerful phosphate solubilizers are from the genera *Bacillus*, *Rhizobium* and *Pseudomonas* and nonsymbiotic nitrogen-fixing bacteria such as *Azotobacter* and the nitrogen-fixing spirochete *Azospirillum* (Saharan and Nehra, 2011). Symbiotic strains also have the ability to directly inhibit the growth of pathogenic bacteria (Ni et al., 2019), which helps to protect plants from certain pathogens. Most of the symbiotic strains obtained in this study had more than one growth-promoting function characteristic. By screening the symbiotic strains for *in vitro* growth-promoting and plate antagonistic functions, four strains of symbiotic strains with strong growth-promoting and antagonistic functions were obtained, two from the interrhizosphere and two from endophytes. Phylogenetic analysis showed that the four strains were TPD11 (*Aspergillus tamarii*), TPB (*Fusarium oxysporum*), LgD10 (*Bacillus thuringiensis*) and LgD2 (*Bacillus cereus*), and their growth-promoting effects on the content of parissaponin and their effects on the control of tobacco black shank were investigated.

## 4.2 The effect of probiotic strains on host plants

The growth of *P. polyphylla* var. *yunnanensis* was promoted by the growth-promoting antagonistic strains obtained in this study, mainly in terms of biomass, photosynthesis and chlorophyll. According to previous reports, inoculation with probiotic bacteria can promote the growth of host plants and increase biomass, photosynthetic efficiency and chlorophyll content (Chen et al., 2005; Nautiyal et al., 2008; Naveed et al., 2014). Two symbiotic strains of bacteria isolated from cucumber roots by Waqas et al. (2012) significantly increased the branch length, chlorophyll content, fresh weight and dry weight of rice. In this study, inoculation with four strains of probiotic bacteria increased the plant height, root weight, aboveground fresh weight, chlorophyll content, net photosynthetic rate, stomatal conductance, and transpiration rate of *P. polyphylla* var. *yunnanensis* to different degrees.

Some symbiotic strains have the potential to produce biosynthesis of the same or similar metabolites present in the host plant, in addition to promoting plant growth. Bashyal et al. (1999) isolated the endophytic bacterium *Seimatoantlerium nepalense* from redbud to produce paclitaxel, and Chithra et al. (2014) isolated *Colletotrichum gloeosporioides* from Piperaceae that had the ability to form piperine. In the present study, the symbiotic strains TPD11, TPB, LgD10 and LgD2 promoted the accumulation of total saponins of *P. polyphylla* var. *yunnanensis* to different degrees.

Previous studies have suggested that upregulation of genes related to the secondary metabolite synthesis pathway may contribute to the increased secondary metabolite content (Alam et al., 2021). Cui et al. (2017) isolated the endophytic fungus *Trimmatostroma* sp. from red buttercup that could induce the accumulation of rhodopsin and tyrosol by regulating the expression activity of key enzyme genes. The endophytic fungus *Paraconiothyrium* SSM001 (Soliman et al., 2013) enhances paclitaxel content in host plants by upregulating the genes *TS*, *DXR*, and *HMGR*, which are related to paclitaxel synthesis. Mevalonate (*MVA*) is the main pathway for the synthesis of steroidal saponins, and *HMGS*, *GPPS*, *FPPS*, *SS*, *CAS*, and *SE* are key genes in the *MVA* pathway (Mishra et al., 2016; Yang et al., 2017), so it is important to study the expression of related genes for saponin synthesis. We showed that the expression levels of *HMGS*, *GPPS*, *FPPS*, *SS*, *CAS* and *SE* genes were significantly upregulated after inoculation with probiotic bacteria, with the highest expression of *HMGS*, *SE2* and *CAS* under the LgD10 strain treatment, the highest expression of *GGPS* and *FPPS* under the TPD11 strain treatment, and the highest expression of *SE1* under the LgD2 treatment.

## 4.3 Effect of inoculation with growth-promoting strains on tobacco

Symbiotic strains can indirectly improve plant growth by resisting harmful pathogens or inhibiting the growth of pathogenic bacteria in a variety of ways, reducing the impact of pests and diseases on plant growth and development. Gang et al. (Gang, 1992) inoculated cucumber seeds with probiotic antagonistic bacteria, which significantly reduced the anthracnose of cucumber leaves compared to the uninoculated control. Silosuh et al. (Silo-Suh et al., 1994) isolated a strain of *Bacillus cereus* UW85 that controlled alfalfa damping-off by inhibiting oomycete pathogens and producing two antimicrobial substances, aminopolyols and aminoglycosides. Baskar et al. (2020) isolated and identified an endophytic fungus from the cactus *Aspergillus tamarii* based on the fact that its mycelial metabolite ethyl acetate has a good insecticidal effect. An efficient system for the bioconversion of sucrose to oligofructose (FOS) was obtained using *Aspergillus tamarii* NKRC 1229 mycelial fructosyltransferase (Choukade and Kango, 2019). Akhtar

(Akhtar et al., 2021) isolated 15 strains from heavy metal-contaminated soil and screened them for heavy metal tolerance and plant promotion potential to identify the most efficient strain, *Bacillus cereus*. Significantly improved plant growth and reduced chromium toxicity after inoculation with *Bacillus cereus* also improved photosynthetic pigments, i.e., chlorophyll a, chlorophyll b, chlorophyll a+b and carotenoids also increased plant superoxide dismutase activity SOD. Fravel et al. (2003) showed that the nonpathogenic *Fusarium oxysporum* shows several modes of action that contribute to its biocontrol capacity. They can compete for nutrients in the soil and thus affect the germination rate of thick-walled spores of the pathogen. They can also compete for infection sites in the roots and can trigger plant defense responses and induce systemic resistance. *Arabidopsis thuringiensis* reduced the levels of cytokinin, gibberellin, jasmonic acid and abscisic acid and increased the levels of indole-3-acetic acid IAA and salicylic acid SA after 24 h of treatment with *Bacillus thuringiensis* (Subramanian, 2019). Jung et al. (2008) treated *Bacillus thuringiensis* to increase phenolic production, phenylalanine deaminase activity (a lignification-related enzyme) and levels of peroxidase and superoxide dismutase (antioxidant enzymes) in 2-week-old soybean plants, indicating that it stimulated defense system responses. In this study, the biocontrol ability of four strains of probiotic bacteria and fungi was determined, and pathogenic fungal antagonism experiments and pot tests showed that all four strains of probiotic antagonistic bacteria inhibited the growth of tobacco black shank, significantly reduced the incidence and disease index of tobacco black shank and promoted plant growth. The most significant effects of the TPD11+Y and LgD10+Y treatments were observed in the antagonistic group.

Biotrophic bacteria were shown to be able to promote host plant growth and disease resistance by inducing defense enzyme activities, similar to the mechanism of innate plant disease resistance. Polyphenol oxidase (PPO) and phenylalanine aminolase (PAL) are secondary metabolism-related enzymes, and the higher the PPO and PAL activities of the plant are, the greater its disease resistance (Schneider et al., 1994). The synergistic action of superoxide dismutase (SOD) and peroxidase (POD) can scavenge superoxide radicals and prevent peroxide-induced cell membrane damage (Benliang et al., 2019). Wang et al. (2020) found that inoculation with *Pseudomonas aeruginosa* strain CQ-4 significantly promoted tomato seed germination and seedling growth and induced upregulation of the activities of four disease-resistant defense enzymes (PPO, POD, SOD and PAL) in plants, thereby effectively controlling *Botrytis cinerea*. Chowdappa (Chowdappa et al., 2013) reported increased host enzyme activities induced by *Bacillus subtilis*, including POD, PPO and SOD, and various hormone synthesizing enzymes to induce ISR against early and late blight of tomato seedlings.

### 4.3 Effect of probiotic strains on the expression of related genes

In this study, inoculation with four strains of probiotic antagonist bacteria promoted the activity of tobacco PPO, POD, SOD and PAL defense enzymes to different degrees, and the probiotic group of TPD11 and LgD10 strains had the most significant effect on defense enzyme activity. Previous studies have shown that the *NtPPO*, *PAL*, *NtSOD* and *NbDADIR* genes are genes that control the synthesis of four enzymes, PPO, PAL, SOD and POD, respectively (Li et al., 2020); the *NbRbohA* and *NbRbohB* genes play an important role in tobacco resistance to *Phytophthora nicotianae* infection (Yoshioka et al., 2003); and the *NtHSR203J*, *NtTTG2* and *NtPRIa* genes are associated with growth and disease resistance in tobacco (Li et al., 2012; Xu et al., 2013). In the present study, these nine genes were analyzed in terms of disease resistance performance and growth promotion, and only the expression of these nine genes was upregulated in the inoculated TPD11 and LgD10 strain treatments compared to the control, while the rest of the genes were not expressed in the strain treatments.

In summary, in this study, a total of 73 strains of symbiotic bacteria were isolated from the rhizomes and interrhizosphere soil of *P. fargesii* Franch. A comprehensive analysis of the disease-promoting effects of symbiotic strains shows that four strains, TPD11, TPB, LgD10 and LgD2, were found to have significant effects on regulating the growth of *P. polyphylla* var. *yunnanensis*, promoting the accumulation of saponin VI and the disease resistance of tobacco.

### Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank, accession numbers OP404241, OP404360, OP405007 and OP405014.

### Author contributions

LT conceived and designed the experiments and wrote this paper. DY and XW analyzed the data. CX and SZ prepared the plant materials. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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